

CLAIMS

What is claimed is:

1. A method for detecting *Listeria spp.* in a sample, the method comprising:
 - (a) providing an inert surface having adhered thereto anti-*Listeria* antibodies capable of capturing *Listeria spp.* cells;
 - (b) contacting the surface of step (a) with a sample suspected of containing *Listeria spp.*, wherein *Listeria spp.* cells present in the sample adhere to the anti-*Listeria* antibodies on the surface;
 - (c) contacting the surface of step (b) with a substrate for beta-glucosidase that produces luminescence when hydrolyzed, wherein beta-glucosidase produced by the *Listeria spp.* cells adhered to the anti-*Listeria* antibodies catalyzes hydrolysis of the substrate; and
 - (d) contacting the surface of step (c) with an enhancer molecule, and then
 - (e) detecting the luminescence generated in step (c), wherein the luminescence is indicative of the presence of the *Listeria spp.* cells in the sample.
2. The method of Claim 1, wherein in step (a), the inert surface is a particle.
3. The method of Claim 1, wherein in step (a), the inert surface is a magnetic particle.
4. The method of Claim 1, wherein in step (a), the inert surface is a silica-coated particle.
5. The method of Claim 1, wherein in step (a), the inert surface is a dextran-coated particle.
6. The method of Claim 1, wherein in step (a), the inert surface is a silica- and dextran-coated particle.

7. The method of Claim 1, wherein in step (a), the inert surface has adhered thereto anti-Listeria IgG.

8. The method of Claim 1, wherein in step (c), the substrate for beta-glucosidase comprises a 1,2-dioxetane.

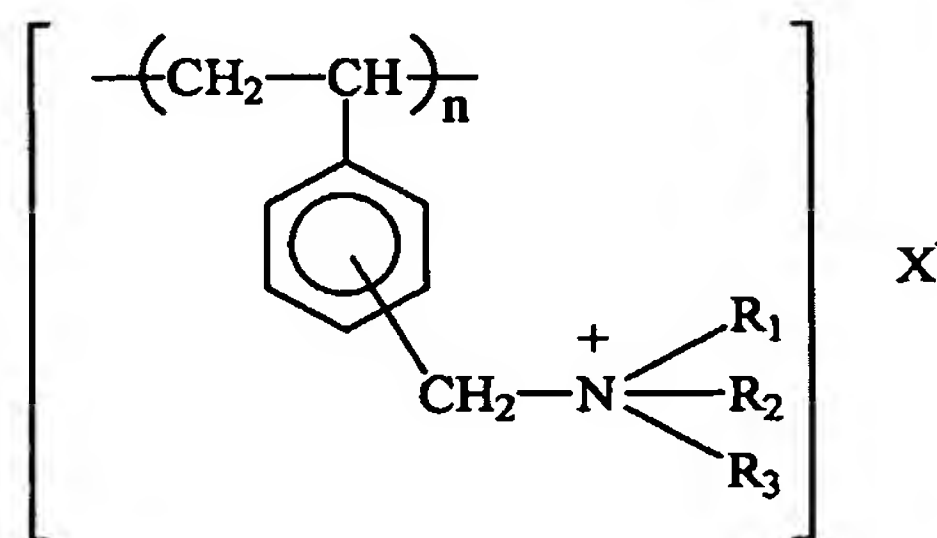
9. The method of Claim 8, wherein in step (c), the substrate for beta-glucosidase comprises a compound selected from the group consisting of {(4-(2-phenoxyethoxy)-4-(3-phosphoryloxy-4-chlorophenyl)) spiro {1,2-dioxetane-3,13'-tricyclo{7.3.1.0^{2,7}}tridec-2,7-ene} and salts thereof.

10. The method of Claim 1, wherein in step (d), the enhancer molecule comprises a co-polymer of styrene and a polymerizable quaternary ammonium monomer.

11. The method of Claim 1, wherein in step (d), the enhancer molecule comprises a poly(vinylbenzyl) ammonium polymer having an weight average molecular weight (M_w) of from about 50,000 to 70,000 Da.

12. The method of Claim 1, wherein in step (d), the enhancer molecule is selected from the group consisting of compounds of Formula I and Formula II:

Formula I:



Formula I

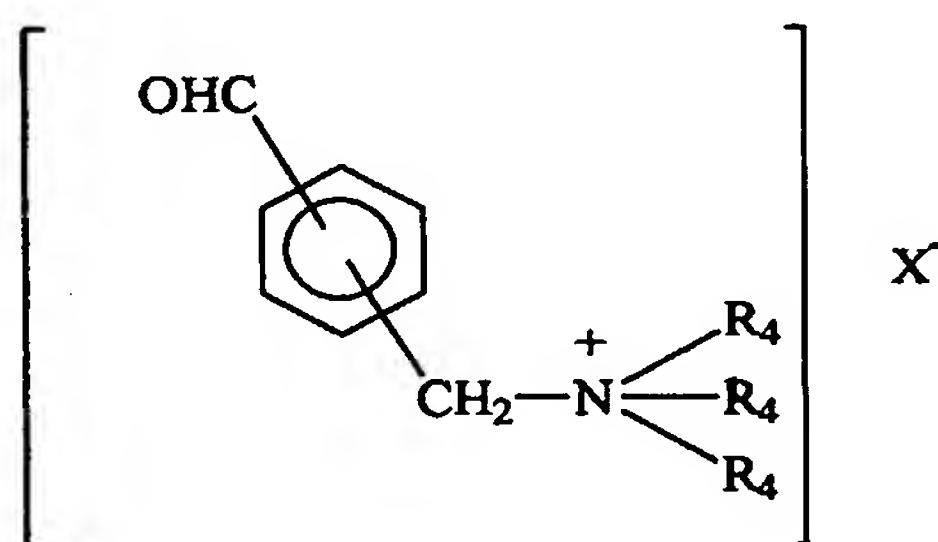
wherein each of R_1 , R_2 and R_3 can be a straight or branched chain unsubstituted alkyl group having from 1 to 20 carbon atoms, a straight or branched chain alkyl group having from 1 to 20 carbon atoms substituted with one or more hydroxy, alkoxy, aryloxy, amino, substituted amino, amido, fluoroalkane, or fluoroaryl groups; an unsubstituted monocycloalkyl group having from 3 to 12 ring

carbon atoms, a substituted monocycloalkyl group having from 3 to 12 ring carbon atoms substituted with one or more alkyl, alkoxy or fused benzo groups; a polycycloalkyl group having 2 or more fused rings, each having from 5 to 12 carbon atoms unsubstituted or substituted with one or more alkyl, alkoxy or aryl groups; an aryl, alkaryl or aralkyl group having at least one ring and from 6 to 20 carbon atoms in toto, unsubstituted or substituted with one or more alkyl, aryl, or fluoroalkane or fluoroaryl groups;

X- is a counterion; and

"n" is a positive integer such that the molecular weight of the Formula I compound will range from about 800 to about 200,000 Da; and

water-soluble acetals of a polyvinylalcohol and a formylbenzyl quaternary ammonium salt as shown in Formula II:



Formula II

wherein each R₄ is the same or a different aliphatic substituent and X- is an anion.

13. The method according to any one of Claims 1 to 12, further comprising, prior to step (c) aging the substrate at room temperature in the presence of proteins for a period of at least 12 hours.

14. The method according to Claim 13, comprising aging the substrate at room temperature for a period of at least 24 hours.

15. The method according to Claim 13, comprising aging the substrate at room temperature for a period of at least 48 hours.

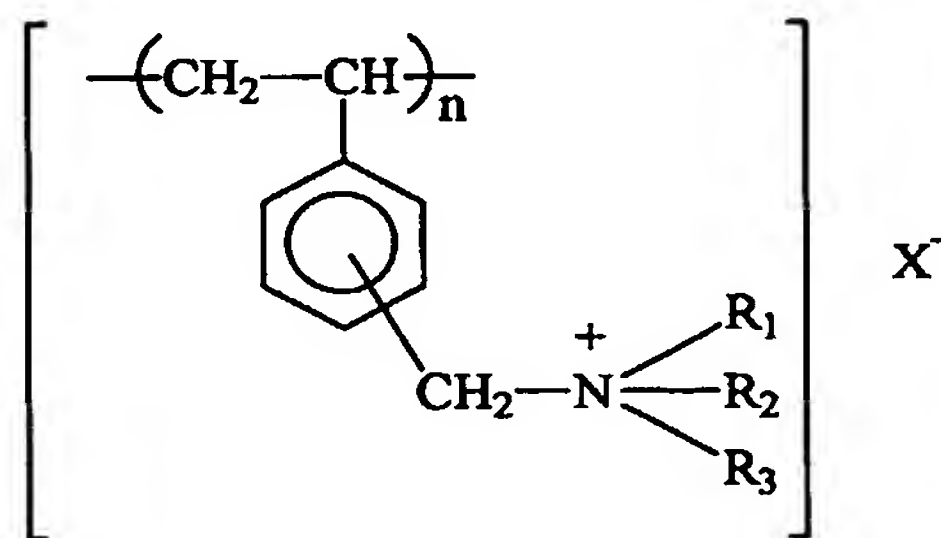
16. The method according to Claim 13, comprising aging the substrate in the presence of heat-denatured proteins.
17. The method according to any one of Claims 1 to 12, further comprising, after step (b) and prior to step (c), separating the surface from the sample.
18. A kit for detecting *Listeria spp.* in a sample, the kit comprising:
 - an inert surface having adhered thereto anti-*Listeria* antibodies capable of capturing *Listeria spp.* cells;
 - a substrate for beta-glucosidase that produces luminescence when hydrolyzed, wherein the substrate is disposed in a first container;
 - an enhancer molecule disposed in a second container; and
 - instructions for use of the kit.
19. The kit of Claim 18, wherein the inert surface is a particle.
20. The kit of Claim 18, wherein the inert surface is a magnetic particle.
21. The kit of Claim 18, wherein the inert surface is a silica-coated particle.
22. The kit of Claim 18, wherein the inert surface is a dextran-coated particle.
23. The kit of Claim 18, wherein the inert surface is a silica- and dextran-coated particle.
24. The kit of Claim 18, wherein the inert surface has adhered thereto anti-*Listeria* IgG.
25. The kit of Claim 18, wherein the substrate for beta-glucosidase comprises a 1,2-dioxetane.

26. The kit of Claim 18, wherein the substrate for beta-glucosidase comprises a compound selected from the group consisting of {(4-(2-phenoxyethoxy)-4-(3-phosphoryloxy-4-chlorophenyl))} spiro {1,2-dioxetane-3,13'-tricyclo{7.3.1.0^{2,7}}tridec-2,7-ene} and salts thereof.

27. The kit of Claim 18, the enhancer molecule comprises a co-polymer of styrene and a polymerizable quaternary ammonium monomer.

28. The kit of Claim 18, wherein the enhancer molecule comprises a poly(vinylbenzyl) ammonium polymer having an weight average molecular weight (M_w) of from about 50,000 to 70,000 Da.

29. The kit of Claim 18, wherein the enhancer molecule is selected from the group consisting of compounds of Formula I:



Formula I

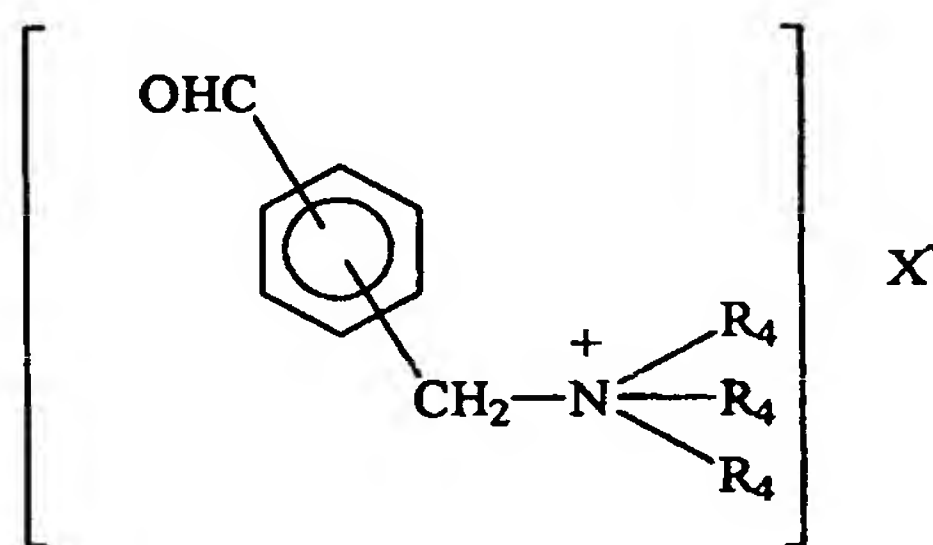
wherein each of R_1 , R_2 and R_3 can be a straight or branched chain unsubstituted alkyl group having from 1 to 20 carbon atoms, a straight or branched chain alkyl group having from 1 to 20 carbon atoms substituted with one or more hydroxy, alkoxy, aryloxy, amino, substituted amino, amido, fluoroalkane, or fluoroaryl groups; an unsubstituted monocycloalkyl group having from 3 to 12 ring carbon atoms, a substituted monocycloalkyl group having from 3 to 12 ring carbon atoms substituted with one or more alkyl, alkoxy or fused benzo groups; a polycycloalkyl group having 2 or more fused rings, each having from 5 to 12 carbon atoms unsubstituted or substituted with one or more alkyl, alkoxy or aryl groups; an aryl, alkaryl or aralkyl group having at least one ring and from 6 to 20 carbon atoms

in toto, unsubstituted or substituted with one or more alkyl, aryl, or fluoroalkane or fluoroaryl groups;

X⁻ is a counterion; and

"n" is a positive integer such that the molecular weight of the Formula I compound will range from about 800 to about 200,000 Da; and

water-soluble acetals of a polyvinylalcohol and a formylbenzyl quaternary ammonium salt as shown in Formula II:



Formula II

wherein each R₄ is the same or a different aliphatic substituent and X⁻ is an anion.